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Multiple origins of viviparity, or reversal from viviparity to oviparity? The European common lizard (*Zootoca vivipara*, Lacertidae) and the evolution of parity

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The evolution of viviparity in squamates has been the focus of much scientific attention in previous years. In particular, the possibility of the transition from viviparity back to oviparity has been the subject of a vigorous debate. Some studies have suggested this reversal is more frequent than previously thought. However, none of them provide conclusive evidence. We investigated this problem by studying the phylogenetic relationships between oviparous and viviparous lineages of the reproductively bimodal lizard species *Zootoca vivipara*. Our results show that viviparous populations are not monophyletic, and that several evolutionary transitions in parity mode have occurred. The most parsimonious scenario involves a single origin of viviparity followed by a reversal back to oviparity. This is the first study with a strongly supported phylogenetic framework supporting a transition from viviparity to oviparity. © 2006 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2006, **87**, 1–11.

ADDITIONAL KEYWORDS: evolution of viviparity – mtDNA – phylogeny.

INTRODUCTION

Squamates are an ideal system for the study of the evolution of reproductive modes. Indeed, phylogenetic analyses indicate that evolutionary transitions from oviparity to viviparity have occurred more often in squamates than in all other lineages of vertebrates combined (Blackburn, 1982, 1985, 1999; Shine, 1985). However, this conclusion may be biased because it relies on the assumption that viviparity is derived from oviparity, and that the reverse transition is rare or impossible (Tinkle & Gibbons, 1977). This traditional assumption has been challenged recently by de Fraipont, Clobert & Barbault (1996) and de Fraipont *et al*. (1999), who enumerated several evolutionary

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*Corresponding author. Current address: School of Biological Sciences, University of Wales, Bangor LL57 2UW, UK. E-mail: y.surget-groba@bangor.ac.uk transitions from viviparity to oviparity and suggested that this transition was much more frequent than previously thought. Subsequent authors have suggested the existence of such reversals in various squamate taxa (Benabib, Kjer & Sites, 1997; Schulte *et al*., 2000; Smith, Austin & Shine, 2001). However, none of these studies provide conclusive evidence for the evolution of oviparity from viviparity because they lack well-supported phylogenetic evidence. For instance, de Fraipont *et al.* (1996) were criticized because they based their results on poorly supported high-level phylogenies and on comparisons of distantly related taxa (Blackburn, 1999; Shine & Lee, 1999). Benabib *et al*. (1997) suffer from errors in assessing the reproductive mode of the taxa studied (Mendez-de la Cruz, Villagran-Santa Cruz & Andrew, 1998). In the study of Schulte *et al*. (2000), different scenarios of reproductive mode transitions were equally parsimonious. Because of its poor support (low bootstrap support (BS) for the main nodes), the phylogeny obtained by Smith *et al*. (2001), though suggesting possibilities of reversion, did not allow rejection of alternative hypotheses.

Hence, there remains a need for empirical research to identify and polarize the evolutionary transitions of reproductive modes in squamate lineages using strongly supported phylogenetic evidence. Single species with reproductive bimodality (i.e. oviparous and viviparous lineages within a single species) are especially informative because the parity transitions are more recent and better allow for the study of microevolutionary mechanisms. Reproductive bimodality has been documented for three lizard species: the Australian scincids *Lerista bougainvilli* (Qualls *et al*., 1995) and *Saiphos equallis* (Smith & Shine, 1997), and the Eurasian lacertid *Zootoca vivipara* (Braña & Bea, 1987; Heulin, 1988).

Zootoca vivipara (formerly *Lacerta vivipara*) is a small lacertid lizard with allopatric and parapatric oviparous and viviparous populations. Most of the range of *Z. vivipara*, from the British Isles and central France into Scandinavia and eastern Russia, is viviparous, whereas two distinct, allopatric oviparous populations are restricted to the southern margin of the range (Fig. 1). The 'western oviparous group' is found in southern France and northern Spain (Heulin & Guillaume, 1989), and the 'eastern oviparous group' is located in northern Italy, southern Austria, Slovenia and Croatia (Heulin *et al*., 2000; Ghielmi *et al*., 2001). We have previously shown that eggs from the eastern oviparous group have thicker shells and contain embryos less developed at the time of oviposition compared with the eggs from the western oviparous group (Heulin *et al*., 2002). The embryos of viviparous females have lecitotrophic (from the yolk) nutrition and remain enveloped in a thin eggshell membrane during the entire gestational period (Panigel, 1956; Heulin, 1990).

We previously examined the phylogenetic relationships of several oviparous and viviparous populations of this species (Surget-Groba *et al*., 2001). We identified five distinct clades (Fig. 2), two oviparous clades (eastern and western) and three viviparous clades (central, eastern, and western). Low sequence variation in the mtDNA sequence fragment analysed (429 bp of the cytochrome *b* gene) did not allow us to assess with certainty whether the viviparous populations were monophyletic: monophyly of the viviparous populations was only weakly supported by a neighbour-joining analysis (BS = 51), while the position of clade C was unresolved using a parsimony analysis (Fig. 2). We concluded, however, that the most conservative hypothesis is that only one origin of viviparity occurred.

In this paper, we present a phylogenetic analysis based on a much larger mtDNA fragment and more comprehensive sampling, including many new populations from Asia and from central Europe, where the greatest part of the genetic diversity resides. The purpose of this research was to study transitions between oviparous and viviparous populations in a phylogenetic context.

MATERIAL AND METHODS

SAMPLES

Tissue samples were available for two outgroup species (*Lacerta bilineata* and *Podarcis muralis*) and for 522 individuals from 142 populations distributed throughout the range of *Z. vivipara* (Appendix, Fig. 2). Direct observations of egg-laying or parturition were obtained for 71 of the populations (Appendix).

MOLECULAR METHODS

DNA was chelex-extracted from tissue samples stored in 95% ethanol. We first determined the haplotype of all 522 of the samples using the same 429-bp fragment (23 bp of Glu-tRNA and 406 bp of cytochrome *b*) previously studied (Surget-Groba *et al*., 2001) by sequencing or single-strand conformation polymorphism (SSCP) analysis (for details, see Surget-Groba *et al*., 2001; Surget-Groba *et al*., 2002). Next, a further 737 bp of cytochrome *b* was sequenced in one representative of each unique haplotype ($N = 48$) to obtain the complete cytochrome *b* gene (1143 bp) as well as about 500 bp of the 16S rRNA gene (between 479 and 484 bp depending on the haplotype). The primers used for the cytochrome b gene were MVZ04, MVZ05 (Smith & Patton, 1991), L15153, L15369, H15488, H15915 (Fu, 2000), CBL392 (ATAGCCA CAGCTTTTTTGG, this study) and CBH878

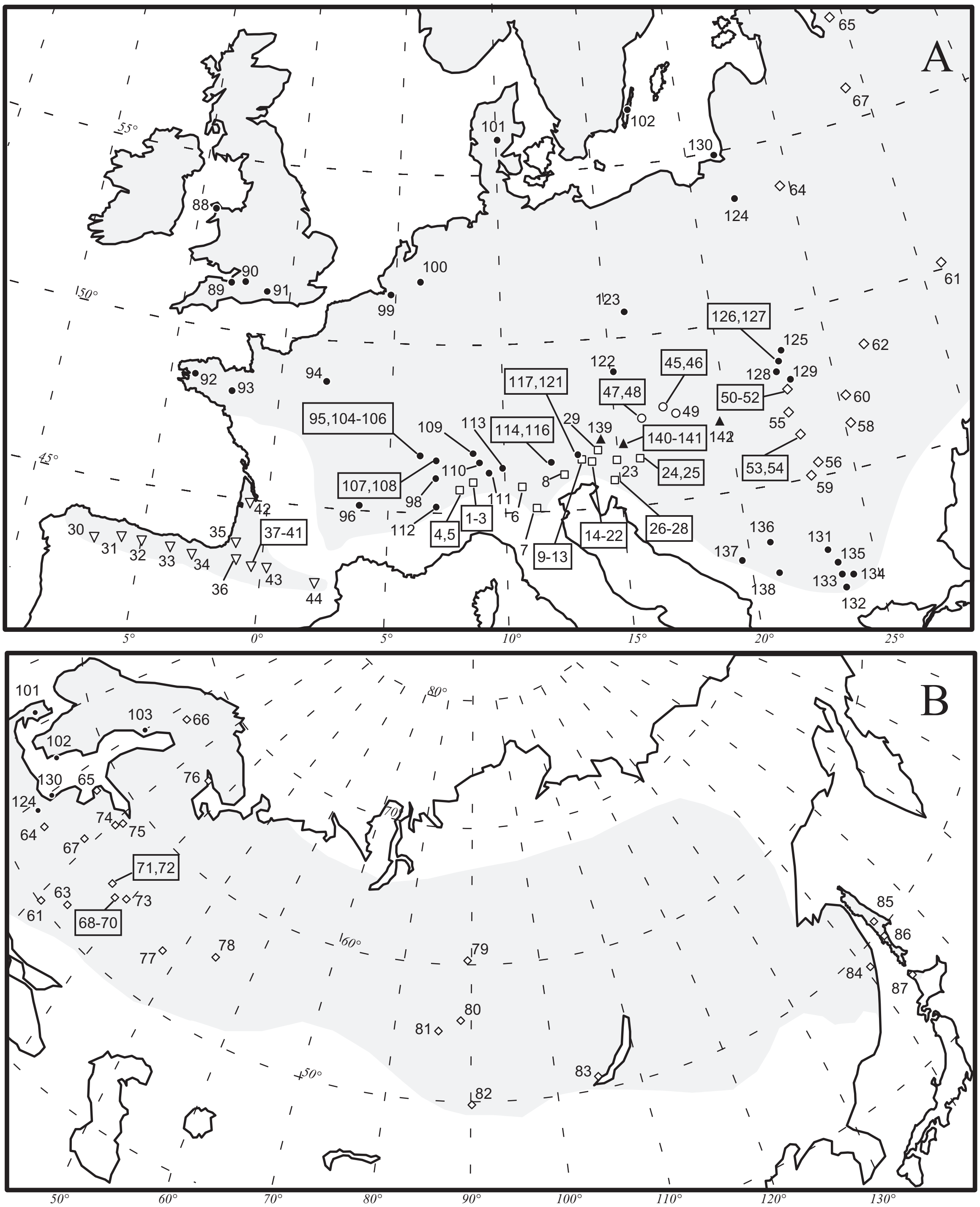

Figure 1. Localization of the sampled populations in (A) European part of the range and (B) Asian part of the range. Broken lines represent parallel and meridian lines. Italic numbers indicate the corresponding latitudes and longitudes. Collection sites are listed in Appendix. Symbols identify the clade to which the population belongs (△, western oviparous clade; □, eastern oviparous clade; ○, first central viviparous clade; ▲, second central viviparous clade; ◇, eastern viviparous clade; ●, western viviparous clade). The shaded area represents the distribution range of the species.

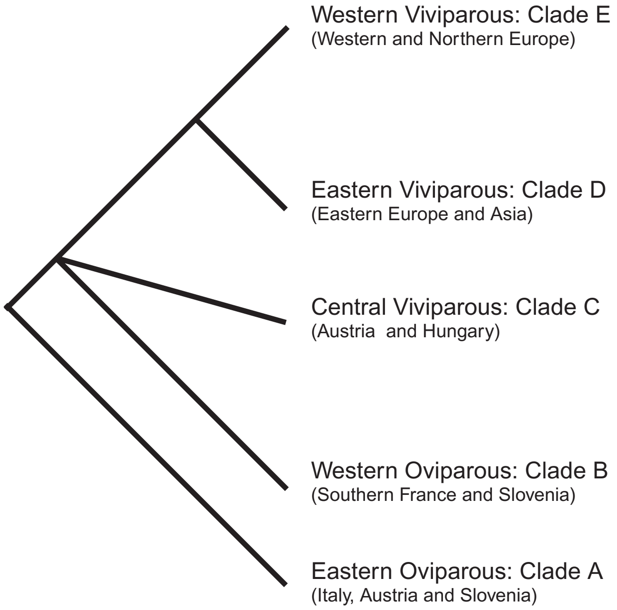

Figure 2. Phylogenetic relationships between the oviparous and viviparous strains of *Zootoca vivipara* according to Surget-Groba *et al*. (2001).

(TTAAATTGAGAATAGAAGAGCC, this study) and for the 16S rRNA gene we used 984 and 986 (Clary & Wolstenholme, 1985). All sequences have been deposited in GenBank (GenBank accession AY714882–AY714981).

PHYLOGENETIC ANALYSES

Sequences were aligned using Sequencher (Gene Codes Corp.). Phylogenetic analyses were performed with PAUP* (Swofford, 2002). Gaps were scored as missing characters. For maximum parsimony analyses, we conducted a heuristic search with 100 replications and TBR branch swapping. Because characters with several changes (homoplasious characters) are unreliable indicators of relationships, we applied the successive weighting method (Farris, 1969; Horovitz & Meyer, 1995) using the maximum value of the rescaled consistency index (RC) for each character. Node support was estimated by 1000 bootstrap replicates (full heuristic search with 10 replications and TBR branch swapping).

RESULTS

PHYLOGENETIC RELATIONSHIPS

The complete dataset consisted of 1660 aligned base pairs: 23 bp of Glu-tRNA, 1143 bp of cytochrome *b*, 4 bp at the 3′ end of the cytochrome *b* gene, and 490 bp of 16S rRNA. Parsimony analysis produced 10 trees of 816 steps (consistency index (CI) = 0.7390; retention index (RI) = 0.8461; RC = 0.6252). The strict consensus of these 10 trees is shown in Figure 3. After successive weighting of the characters according to their RC, six trees were produced (CI = 0.8959; RI = 0.9222; RC = 0.8262). The strict consensus of these trees was identical to the one without weighting except for one terminal node (VB14 and VB15 branched together). Bootstrap support was much better using this weighting procedure (Fig. 3). This reflects the elimination of homoplasic characters that compromise phylogenetic inference.

According to the phylogenetic hypothesis (Fig. 3), we could distinguish two oviparous and four viviparous lineages in *Z. vivipara* (Fig. 3):

1. An eastern oviparous group (Clade A, with seven haplotypes; BS = 100), corresponding to the subspecies *Z. vivipara carniolica* (Mayer *et al*., 2000) from Italy, southern Austria and Slovenia.
2. A western oviparous group (Clade B, with eight haplotypes; BS = 88), distributed in southern France and northern Spain.
3. One viviparous group from central Europe (Clade C, with two haplotypes; BS = 100), corresponding to five populations from north-eastern Austria–north-western Hungary.
4. Another viviparous group from central Europe (Clade F, with two haplotypes; BS = 100), corresponding to four populations from central Hungary and southern Austria.
5. An eastern viviparous group (Clade D, with nine haplotypes; BS = 94), widely distributed in eastern Europe and Asia.
6. A western viviparous group (Clade E, with 20 haplotypes; BS = 99), distributed in western Europe, Bulgaria and Serbia.

All haplotypes from *Z. vivipara* formed a monophyletic group. Haplotypes from the oviparous *Z. v. carniolica* (Clade A) were located at the base of this tree. Two major clades constituted the remainder of the tree: the first clade included the two central viviparous groups (Clades C and F) and the western oviparous group (Clade B), and the second clade included the eastern and western viviparous groups (Clades D and E, respectively). Neither the oviparous nor the viviparous haplotypes formed a monophyletic assemblage. Indeed, the western oviparous clade (Clade B) was the sister group of the first central viviparous clade (Clade C) and this assemblage (Clades B + C) was the sister group of the second central viviparous group (Clade F). The monophyly of these three clades (B + C + F) was strongly supported (BS = 94). The eastern and western viviparous groups formed a monophyletic group (BS = 82).

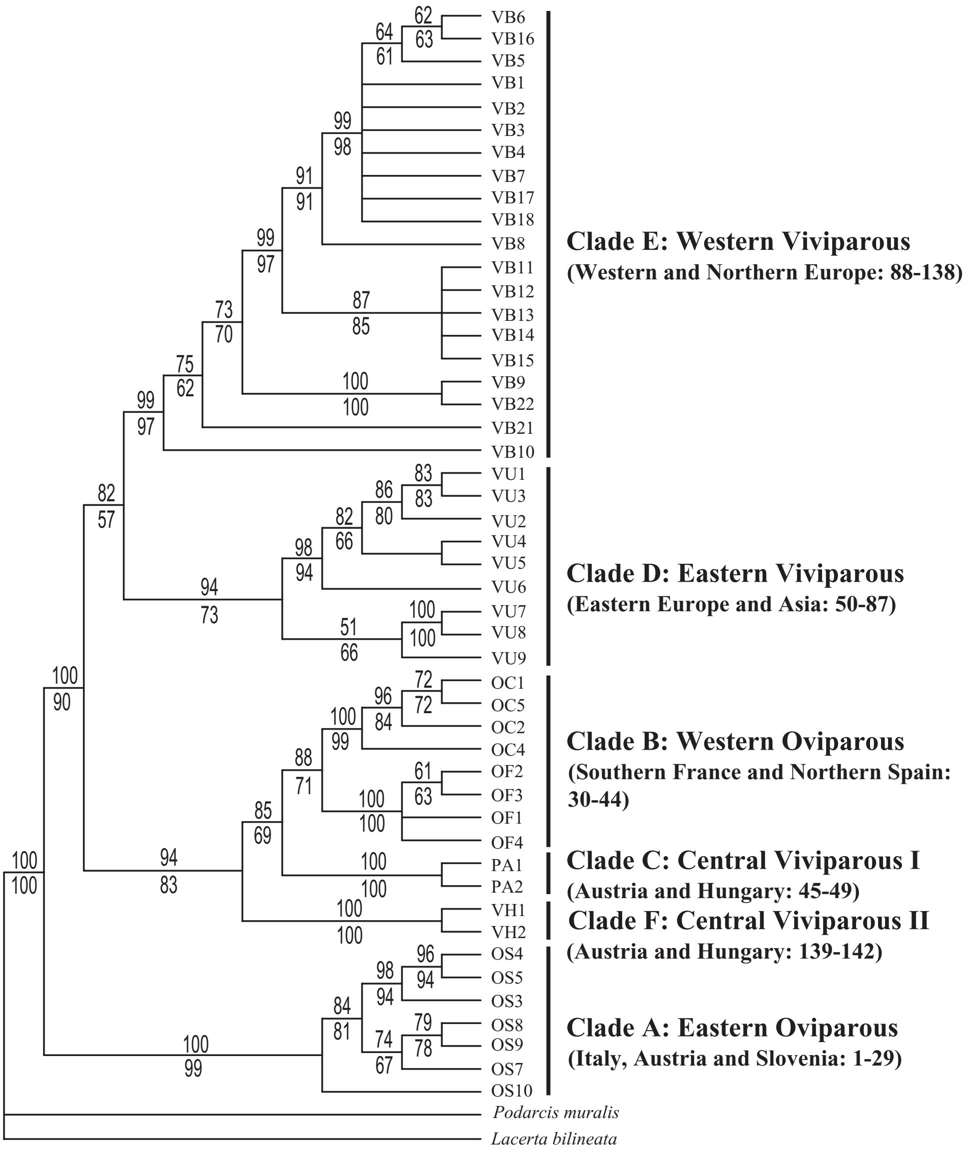

Figure 3. Maximum parsimony strict consensus tree for 48 *Zootoca vivipara* mtDNA haplotypes rooted with *Podarcis muralis* and *Lacerta bilineata*. Names for tip taxa correspond to haplotype names as available in GenBank. Numbers are bootstrap values with (above branches) or without (below branches) successive weighting of characters according to their rescaled consistency index value. Numbers between brackets indicate the populations belonging to each clade (same numbers as in Fig. 1).

DISCUSSION

Compared with our earlier study (Surget-Groba *et al.*, 2001), this study greatly improves the resolution of and support for our phylogenetic hypothesis for *Z. vivipara*. For instance, the viviparous Clade C, whose phylogenetic position was previously unresolved, was supported as the sister clade of the western oviparous populations (Clade B). A newly discovered viviparous lineage in central Europe (Clade F; Fig. 2) also clustered with these two groups. The phylogenetic position of the other clades (A, D and E) remained unchanged.

With regard to the evolution of reproductive modes, we previously suggested that the most conservative (but weakly supported) hypothesis was that the viviparous clades of *Z. vivipara* are monophyletic and therefore that a single origin of viviparity occurred in this species (Surget-Groba *et al.*, 2001). The phylogenetic tree obtained in this study now leads us to reject this hypothesis. In fact, neither the viviparous nor the oviparous populations were monophyletic. Two alternative scenarios for the evolutionary transitions of parity modes in *Z. vivipara* are suggested. The first scenario (Fig. 4A) is that viviparity evolved only once but was followed by a reversal back to oviparity that gave rise to the western oviparous clade (Clade B); the second scenario (Fig. 4B) is that viviparity evolved on three distinct occasions (in Clades C, D + E, and F). The first scenario involves only two evolutionary steps (one transition from oviparity to viviparity and one reversal from viviparity to oviparity) while the second involves three steps (three transitions from oviparity to viviparity). The scenario involving a reversal back to oviparity is therefore the most parsimonious.

Although the criterion of parsimony is an important evolutionary principle, it is nonetheless necessary to examine biological information that supports alternative scenarios (Titus & Larson, 1996; Crawford & Wake, 1998). There is strong phylogenetic evidence that the evolutionary transitions from oviparity to viviparity have occurred very frequently (probably more than 100 times) in squamates (Blackburn, 1982, 1985, 1999; Shine, 1985). Thus, multiple independent origins of viviparity within a single species of squamates are plausible, as proposed in the less parsimonious scenario for *Z. vivipara*. Conversely, the suggestion that oviparity has evolved from viviparity in squamate lineages has generated vigorous debate (de Fraipont *et al*., 1996, 1999; Benabib *et al*., 1997; Mendez-de la Cruz *et al*., 1998; Blackburn, 1999; Shine & Lee, 1999; Schulte *et al*., 2000; Smith *et al*., 2001). The dispute centres on the lack of well-supported phylogenetic evidence, as most authors recognize that the transition from viviparity to oviparity cannot be ruled out on theoretical grounds. In considering the evolution of oviparity from viviparity, it is important to emphasize several aspects of the reproductive biology of squamates. In particular, the intrauterine retention of the developing embryo is not exclusively associated with viviparity: most oviparous squamates retain their eggs in the uterus for periods that, depending on the species, represent 20%–80% of the total embryonic developmental time (Packard, Tracy & Roth, 1977; Blackburn, 1982; Shine, 1983; Xavier & Gavaud, 1986; Heulin, Osenegg & Lebouvier, 1991; Demarco, 1993; Andrew & Mathies, 2000; Heulin *et al*., 2002). Hence, the emergence of viviparity in squamates may be viewed as an endpoint along an egg-retention continuum and not as a discrete novelty requiring dramatic character changes. For example, many species of viviparous squamate retain a thin eggshell membrane enveloping the embryo during gestation (Hoffman, 1970; Guillette & Jones, 1985; Stewart, 1985, 1990; Heulin, 1990; Blackburn, 1993; Guillette, 1993; Qualls, 1996). Similarly, as in oviparous species, most viviparous species of squamate still exhibit lecitotrophic (from the yolk) embryonic nutrition (Panigel, 1956; Yaron, 1985; Blackburn, 1993). These observations indicate that the characteristics essential for oviparity may not be irremediably lost in many viviparous squamates. In addition, the redevelopment of complex characters after their loss has been documented in several organisms. For instance, hind limbs may have 're-evolved' in the fossil snakes *Pachyrhachis* and *Haasiophis* (Tchernov *et al*., 2000), as did wings in stick insects (Whiting, Bradler & Maxwell, 2003). The evolutionary transition from viviparity to oviparity therefore remains biologically reasonable.

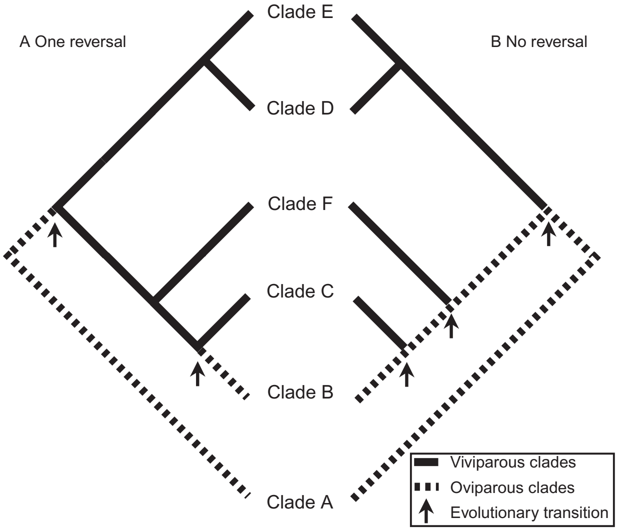

Figure 4. Alternative models explaining the evolution of parity modes in *Zootoca vivipara* considering two hypotheses: A, parity modes are free to reverse; B, the transition from oviparity to viviparity is irreversible.

There is evidence that the evolution of parity modes in squamates is influenced by climatic conditions (viviparous forms favoured under cold conditions, oviparous forms favoured under warmer conditions: for a review see Shine, 1985). As shown previously, the evolutionary history of *Z. vivipara* took place during the Pleistocene (Surget-Groba *et al*., 2001). Hence, the multiple transitions in parity modes in this species could be the consequence of the multiple climatic changes that occurred during this period.

In addition to our data, two other studies based phylogenies of low taxonomic level suggest the occurrence of a transition from viviparity to oviparity. Schulte *et al*. (2000) suggest two equally parsimonious scenarios of either six origins of viviparity or three origins of viviparity followed by three reversals back to oviparity for the evolution of parity modes in the iguanid lizard genus *Liolaemus*. The study on the reproductively bimodal scincid lizard *S. equallis*, though suggesting the possibility of one origin of viviparity followed by one reversal to oviparity, also indicates that a much more conservative hypothesis (implying a single origin of viviparity and no reversal transition) cannot be rejected for this species (Smith *et al*., 2001). This study on *Z. vivipara* is the first with a strongly supported phylogenetic framework indicating a transition from viviparity to oviparity.

The occurrence of multiple (two or three) transitions between oviparity and viviparity in *Z. vivipara* is further evidence that reproductive modes are evolutionarily labile in many squamate lineages. This reproductive instability not only results in variation of the parity mode (oviparous vs. viviparous forms), but also in significant reproductive variation between closely related oviparous forms (see review in Heulin *et al*., 2002). For example, each of the three reproductively bimodal species of lizard (*L. bougainvillii*, *S. equallis* and *Z. vivipara*) exhibit two distinct kinds of oviparity, one with relatively short intrauterine egg-retention (i.e. oviposition of eggs containing less developed embryos) and the other with relatively long intrauterine egg-retention (i.e. oviposition of eggs containing more developed embryos) (Qualls, 1996; Heulin *et al*., 2000, 2002; Smith *et al*., 2001). In addition, a comparative study of the eggshells of the two oviparous forms of *Z. vivipara* and of *L. boungainvillii* revealed that the eggshell is significantly thicker in the form with shorter intrauterine egg retention compared with the form with longer intrauterine egg retention (Qualls, 1996; Heulin *et al*., 2002). For *Z. vivipara,* the oviparous clade (A) with shorter egg retention and thicker eggshells is basally located, whereas the oviparous clade (B) with longer egg-retention and thinner eggshells and the viviparous clades (C, D, E, F) are nested deeper within the tree (see Figs 3, 4). This strongly suggests that the ancestral parity condition of *Z. vivipara* was an oviparous reproductive mode with a relatively short intrauterine retention of egg and with a relatively thick eggshell. This could also be true for *L. bougainvilli* and for *S. equallis*, though this is less-well supported by phylogenetic analyses (Fairbairn *et al*., 1998; Smith *et al*., 2001). Such reproductive variation between different oviparous clades is of considerable interest because they involve the same evolutionary process (variation in intrauterine egg-retention and in eggshell thickness) as those underlying the emergence of viviparity. Future studies of the evolution of parity in squamates should also consider variation in eggshell thickness and in egg retention time within oviparous lineages.

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REFERENCES

Andrews RM, Mathies T. 2000. Natural history of reptilian development: constraints on the evolution of viviparity. *Bioscience* **50:** 227–238.

Benabib M, Kjer KM, Sites JW. 1997. Mitochondrial DNA sequence-based phylogeny and the evolution of viviparity in the *Sceloporus scalaris* group. *Evolution* **51:** 1262–1275.

Blackburn DG. 1982. Evolutionary origins of viviparity in the Reptilia. I. Sauria. *Amphibia-Reptilia* **3:** 185–205.

Blackburn DG. 1985. Evolutionary origins of viviparity in the Reptilia. II. Serpentes, Amphisbaenia, and Ichthyosauria. *Amphibia-Reptilia* **5:** 259–291.

Blackburn DG. 1993. Chorioallantoic placentation in squamate reptiles: structure, function and evolution. *Journal of Experimental Zoology* **266:** 414–430.

Blackburn DG. 1999. Are viviparity and egg-guarding evolutionarily labile in squamates? *Herpetologica* **55:** 556–573.

Braña F, Bea A. 1987. Bimodalité de reproduction chez *Lacerta vivipara*. *Bulletin de la Société Herpétologique de France* **44:** 1–5.

Clary DA, Wolstenholme DR. 1985. The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization and genetic code. *Journal of Molecular Evolution* **22:** 252–271.

Crawford AJ, Wake DB. 1998. Phylogenetic and evolutionary perspectives on an enigmatic organ: the balancer of larval caudate amphibians. *Zoology* **101:** 107–123.

Demarco V. 1993. Estimating egg retention times in sceloporine lizards. *Journal of Herpetology* **27:** 453–458.

Fairbairn J, Shine R, Moritz C, Fommer M. 1998. Phylogenetic relationships between oviparous and viviparous populations of an Australian lizard (*Lerista bougainvillii*). *Molecular Phylogenetics and Evolution* **10:** 95–103.

Farris JS. 1969. A successive approximations approach to character weighting. *Systematic Zoology* **18:** 374–385.

de Fraipont M, Clobert J, Barbault R. 1996. The evolution of oviparity with egg-guarding and viviparity in lizards and snakes: a phylogenetic analysis. *Evolution* **50:** 391–400.

de Fraipont M, Clobert J, Meylan S, Barbault R. 1999. On the evolution of viviparity and egg-guarding in squamate reptiles: a reply to R. Shine and M. S. Y. Lee. *Herpetologica* **55:** 550–555.

Fu JZ. 2000. Towards the phylogeny of the family Lacertidae

– Why 4708 base pairs of mtDNA sequences cannot draw the picture. *Biological Journal of the Linnean Society* **71:** 203–217.

Ghielmi S, Heulin B, Surget-Groba Y, Guillaume C-P. 2001. Identification de populations ovipares de *Lacerta (Zootoca) vivipara* en Italie. *Bulletin de la Société Herpétologique de France* **98:** 19–29.

Guillette L. 1993. The evolution of viviparity in lizards. *Bioscience* **43:** 742–751.

Guillette L, Jones RE. 1985. Ovarian, oviductal and placental morphology of the reproductively bimodal lizard *Sceloporus aeneus*. *Journal of Morphology* **184:** 85–98.

Heulin B. 1988. Données nouvelles sur les populations ovipares de *Lacerta vivipara*. *Comptes Rendus de l'Académie des Sciences de Paris. Série III Sciences de la Vie* **306:** 63–68.

Heulin B. 1990. Etude comparative de la membrane coquillère chez les souches ovipares et vivipares de *Lacerta vivipara*. *Canadian Journal of Zoology* **68:** 1015–1019.

Heulin B, Ghielmi S, Vogrin N, Surget-Groba Y, Guillaume C-P. 2002. Variation in eggshell characteristics and in intra-uterine egg retention between two oviparous clades of the lizard *Lacerta vivipara*: insight into the oviparity-viviparity continuum in Squamates. *Journal of Morphology* **252:** 255–262.

Heulin B, Guillaume C-P. 1989. Extension géographique des populations ovipares de *Lacerta vivipara*. *Revue d'Ecologie* **44:** 39–45.

Heulin B, Guillaume C-P, Vogrin N, Surget-Groba Y, Tadic Z. 2000. Further evidence of the existence of oviparous populations of *Lacerta (Zootoca) vivipara* in the NW of the Balkan Peninsula. *Comptes Rendus de l'Académie des Sciences de Paris. Série III Sciences de la Vie* **323:** 461–468.

Heulin B, Osenegg K, Lebouvier M. 1991. Timing of embryonic development and birth dates in oviparous and viviparous strains of *Lacerta vivipara* – testing the predictions of an evolutionary hypothesis. *Acta Oecologia – Oecologia Generalia* **12:** 517–528.

Hoffman LH. 1970. Placentation in the garter snake: *Thamnophis sirtalis*. *Journal of Morphology* **131:** 57–88.

Horovitz I, Meyer A. 1995. Systematics of New World monkeys (Platyrrhini, Primates) based on 16S mitochondrial sequences: a comparative analysis of different weighting methods in cladistic analysis. *Molecular Phylogenetics and Evolution* **4:** 448–456.

Mayer W, Böhme W, Tiedman F, Bischoff W. 2000. On oviparous populations of *Zootoca vivipara* in south-eastern central Europe and their phylogenetic relationships to neighbouring viviparous and south-west European oviparous populations. *Herpetozoa* **13:** 59–69.

Mendez-de la Cruz FR, Villagran-Santa Cruz M, Andrew R. 1998. Evolution of viviparity in the lizard genus *Sceloporus*. *Herpetologica* **54:** 521–532.

Packard GC, Tracy CR, Roth JJ. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class reptilia. *Biological Reviews* **52:** 71–105.

Panigel M. 1956. Contribution à l'étude de l'ovoviviparité chez les reptiles: gestation et parturition chez le lézard vivipare *Zootoca vivipara*. *Annales de Sciences Naturelles et de Zoologie* **18:** 569–668.

Qualls CP. 1996. Influence of the evolution of viviparity on eggshell morphology in the lizard *Lerista bougainvillii*. *Journal of Morphology* **228:** 119–125.

Qualls CP, Shine R, Donnellan S, Hutchinson M. 1995. The evolution of viviparity within the Australian scincid lizard *Lerista bougainvillii*. *Journal of Zoology* **237:** 13–26.

Schulte JA, Macey JR, Espinoza RE, Larson A. 2000. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. *Biological Journal of the Linnean Society* **69:** 75–102.

Shine R. 1983. Reptilian reproductive modes: the oviparity-viviparity continuum. *Herpetologica* **39:** 1–8.

Shine R. 1985. The evolution of viviparity in reptiles: an ecological analysis. In: Gans C, Billet F, eds. *Biology of the Reptilia*, **Vol. 15.** New York: Wiley, 605–694.

Shine R, Lee MSY. 1999. A reanalysis of the evolution of viviparity and egg-guarding in squamate reptiles. *Herpetologica* **55:** 538–549.

Smith MF, Patton JL. 1991. Variation in mitochondrial cytochrome b sequences in natural populations of South American akodontine rodents (Muridae: Sigmodontinae). *Molecular Biology and Evolution* **8:** 85–103.

Smith SA, Austin CC, Shine R. 2001. A phylogenetic analysis of variation in reproductive mode within an Australian lizard (*Saiphos equalis*, Scincidae). *Biological Journal of the Linnean Society* **74:** 131–139.

Smith SA, Shine R. 1997. Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. *Australian Journal of Zoology* **45:** 435–445.

Stewart JR. 1985. Placentation in the lizard *Gerrhonotus coeruleus* with a comarison to the extraembryonic membranes of the oviparous *Gerrhonotus multicarinatus* (Sauria: Anguidae). *Journal of Morphology* **185:** 101–114.

Stewart JR. 1990. Development of the extraembryonic membranes and histology of the placentae in *Virginia striatula* (Squamata: Serpentes). *Journal of Morphology* **205:** 33–43.

Surget-Groba Y, Heulin B, Ghielmi S, Guillaume C-P, Vogrin N. 2002. Phylogeography and conservation of the populations of *Zootoca vivipara carniolica*. *Biological Conservation* **106:** 365–372.

Surget-Groba Y, Heulin B, Guillaume C-P, Thorpe RS, Kupriyanova L, Vogrin N, Maslak R, Mazzotti S, Venczel M, Ghira I, Odierna G, Leontyeva O, Monney JC, Smith N. 2001. Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. *Molecular Phylogenetics and Evolution* **18:** 449–459.

Swofford DL. 2002. *PAUP: Phylogenetic analysis using parsimony (and other methods)*. Version 4. Sunderland, MA: Sinauer Associates.

Tchernov E, Rieppel O, Zaher H, Polycyn MJ, Jacobs LL. 2000. A fossil snake with limbs. *Science* **287:** 2010–2012.

Tinkle DW, Gibbons JW. 1977. The distribution and evolution of viviparity in reptiles. *Miscellaneous Publications of the University of Michigan* **154:** 1–55.

Titus TA, Larson A. 1996. Molecular phylogenetics of desmognathine salamanders (Caudata: Plethodontidae): a reevaluation of evolution in ecology, life history, and morphology. *Systematic Biology* **45:** 451–472.

Whiting MF, Bradler S, Maxwell T. 2003. Loss and recovery of wings in stick insects. *Nature* **421:** 264–267.

Xavier F, Gavaud J. 1986. Oviparity-viviparity continuum in reptiles; physiological characteristics and relation with environment. In: Assenmacher I, Boissin J, eds. *Endocrine regulation as adaptive mechanisms to the environment*. Paris: CNRS Press, 79–93.

Yaron Z. 1985. Reptilian placentation and gestation: structure, function and endocrine control. In: Gans C, Billet F, eds. *Biology of the Reptilia*, Vol. 15. New York: Wiley, 527–603.

APPENDIX

Collection locality, identification (ID, same as in Fig. 1), sample size (N), reproductive mode and mtDNA clade for all samples included in this study

Clade	ID	Population	*N*	Country
A (Oviparous)	1	Varese*	12	Italy
	2	Mottarone*	11	Italy
	3	Valle Strona†	1	Italy
	4	Oropa*	19	Italy
	5	Valle Sorba†	6	Italy
	6	Bollone*	3	Italy
	7	Busatello†	3	Italy
	8	Cansiglio*	3	Italy
	9	Cavazzo*	2	Italy
	10	Ligosullo	1	Italy
	11	Rio Alba*	2	Italy
	12	Pian Tapou*	1	Italy
	13	Stampoden*	2	Italy
	14	Nordio Deffar	3	Italy
	15	Cave del Predil	1	Italy
	16	Lago del Predil	2	Italy
	17	Fusine*	11	Italy
	18	Valle Bartolo*	7	Italy
	19	Ratece	1	Italy
	20	Valle Saissera*	7	Italy
	21	Podkoren-Zelenci*	10	Slovenia
	22	Veliki Mangart	2	Slovenia
	23	Pavlizevo sedlo	1	Slovenia
	24	Pohorje-Kot*	10	Slovenia
	25	Medvece*	7	Slovenia
	26	Cerknisko Jezero*	9	Slovenia
	27	Ig*	2	Slovenia
	28	Rakov Skocjan	3	Slovenia
	29	Waissach*	3	Austria
B (Oviparous)	30	Puerto de Ancares	1	Spain
	31	Puerto de Letariegos†	1	Spain
	32	Puerto de Tarna†	1	Spain
	33	Alto de Tornos†	2	Spain
	34	Alto de Barazar†	1	Spain
	35	Moura de Montrol*	3	France
	36	Iraty	2	France
	37	Pourtalet*	6	France
	38	Gabas*	21	France
	39	Louvie*	17	France
	40	Benou*	1	France
	41	Plateau de Ger	1	France
	42	St Raphael*	2	France
	43	Col des Palomières*	3	France
	44	Pinet-Belestat	3	France

APPENDIX *Continued*

Clade	ID	Population	*N*	Country
C (Viviparous)	45	Wiener am See*	2	Austria
	46	Moosbrunn	2	Austria
	47	Semmering	2	Austria
	48	Breitenstein	1	Austria
	49	Makotabödöge	4	Hungary
D (Viviparous)	50	Batorliget*	2	Hungary
	51	Fabianhaza	1	Hungary
	52	Mand-Fulesd*	7	Hungary
	53	Apuseni*	5	Romania
	54	Poiana Florilor	5	Romania
	55	Marghita*	4	Romania
	56	Sureanu*	8	Romania
	57	Valdeasa*	6	Romania
	58	Eremitu	5	Romania
	59	Retezat*	13	Romania
	60	Rodnei*	4	Romania
	61	Kiev	1	Ukrainia
	62	Cernovits	2	Ukrainia
	63	Chervonnyy	1	Ukrainia
	64	Grodno*	1	Bielaruss
	65	Matsalu	1	Estonia
	66	Kiruna†	1	Sweeden
	67	Nischa*	1	Russia
	68	Borovsk	1	Russia
	69	Tchekchov	2	Russia
	70	Vostrjakvo	2	Russia
	71	Shahovskoe	1	Russia
	72	Volokolamsk	2	Russia
	73	Chernogolovka	2	Russia
	74	Tolmachevo*	1	Russia
	75	Krasnitsy*	1	Russia
	76	Srednii*	2	Russia
	77	Tschuvachia	1	Russia
	78	Idjevsk*	1	Russia
	79	Turukchanskii Krai	4	Russia
	80	Zaria	1	Russia
	81	Tomsk	1	Russia
	82	Kara-Khol*	1	Russia
	83	Irkutsk*	1	Russia
	84	Grossevithchi	1	Russia
	85	Sakhaline 1	2	Russia
	86	Sakhaline 2	1	Russia
	87	Wakkanai†	2	Japan
E (Viviparous)	88	Anglesey	3	England
	89	Bristol 1†	4	England
	90	Bristol 2†	1	England
	91	Winchester	4	England
	92	St Rivoal	2	France
	93	Paimpont*	31	France
	94	Rambouillet	1	France
	95	Bonnevaux*	13	France
	96	Mas de la Barque†	6	France
	97	Chambery	3	France

APPENDIX *Continued*

Clade	ID	Population	*N*	Country
	98	Vallorcine	1	France
	99	Kalmthout†	10	Belgium
	100	Overasseltse-Haterste Vennen†	1	Netherlands
	101	Them*	1	Denmark
	102	Runsten	1	Sweeden
	103	Umea	1	Sweeden
	104	Chalet à Roch	1	Switzerland
	105	Chatel Saint Denis	1	Switzerland
	106	Vevey*	16	Switzerland
	107	Brassus	2	Switzerland
	108	Charbonnières	3	Switzerland
	109	Hochainplangen	3	Switzerland
	110	Valle Piumogna	2	Switzerland
	111	Valle Morobia	3	Switzerland
	112	Moncenisio	1	Italy
	113	Chiareggio	1	Italy
	114	Valle San Nicolo	1	Italy
	115	Passo Giau	1	Italy
	116	Passo Pordoi	1	Italy
	117	Forni Avoltri	1	Italy
	118	Pian delle Streghe*	2	Italy
	119	Passo di Lanza*	2	Italy
	120	Passo Pramollo*	2	Italy
	121	Hausalm*	1	Austria
	122	Trebon	1	Czech Republic
	123	Szklarska Poreba	10	Poland
	124	Krutyn	10	Poland
	125	Ustrzyki Gorne	12	Poland
	126	Kolonica*	7	Slovakia
	127	Potosna	1	Slovakia
	128	Botany*	11	Slovakia
	129	Tarpa	3	Hungary
	130	Rybachii*	2	Russia
	131	Balkan-Petrohan*	4	Bulgaria
	132	Pirin*	3	Bulgaria
	133	Rila-Belli Iskar*	4	Bulgaria
	134	Rila-Govedarci*	3	Bulgaria
	135	Vitocha*	2	Bulgaria
	136	Kopaonik	1	Serbia
	137	Bjelasica	1	Montenegro
	138	Sara Mount†	2	Kosovo
F (Viviparous)	139	Emberger Alm*	5	Austria
	140	Godingberg	2	Austria
	141	Turracher Höhe	1	Austria
	142	Osca*	5	Hungary

The populations whose reproductive mode was observed are indicated by * (observations of the authors) or † (observations of the collaborators who provided the samples).